



# PATENT COOPERATION TREATY

## PCT

### INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 16894	<b>FOR FURTHER ACTION</b>		See Form PCT/PEA/416
International application No. PCT/CA2004/000998	International filing date (day/month/year) 08.07.2004	Priority date (day/month/year) 08.07.2003	
International Patent Classification (IPC) or national classification and IPC C12N5/06, A01K67/027			
Applicant MCGILL UNIVERSITY et al.			
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 7 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p>a. <input checked="" type="checkbox"/> sent to the applicant and to the International Bureau) a total of 5 sheets, as follows:</p> <p><input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p><input checked="" type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p>b. <input type="checkbox"/> (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>			
<p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the opinion</p> <p><input type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input type="checkbox"/> Box No. VI Certain documents cited</p> <p><input type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input type="checkbox"/> Box No. VIII Certain observations on the international application</p>			
Date of submission of the demand  06.05.2005		Date of completion of this report  21.10.2005	
Name and mailing address of the international preliminary examining authority:  European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016		Authorized Officer  Brouns, G  Telephone No. +31 70 340-3789 	

INTERNATIONAL PRELIMINARY REPORT  
ON PATENTABILITYInternational application No.  
PCT/CA2004/000998

IAP20 Rec'd PCT/PTO 30 DEC 2005

**Box No. I Basis of the report**

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

- ☐ This report is based on translations from the original language into the following language, which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3 and 23.1(b))
  - ☐ publication of the international application (under Rule 12.4)
  - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements\*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

**Description, Pages**

1-33 as originally filed

**Claims, Numbers**

1-26 filed with the demand

**Drawings, Sheets**

1/14-14/14 as originally filed

- ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/figs
- ☐ the sequence listing (*specify*):
- ☐ any table(s) related to sequence listing (*specify*):

4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- ☐ the description, pages
- ☒ the claims, Nos. 4,16,17
- ☐ the drawings, sheets/figs
- ☐ the sequence listing (*specify*):
- ☐ any table(s) related to sequence listing (*specify*):

\* If item 4 applies, some or all of these sheets may be marked "superseded."

**INTERNATIONAL PRELIMINARY REPORT  
ON PATENTABILITY**

International application No.  
PCT/CA2004/000998

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**Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

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**1. Statement**

Novelty (N)	Yes: Claims	1,2,4,5,16-21
	No: Claims	3,6-15,22-26
Inventive step (IS)	Yes: Claims	-
	No: Claims	1-26
Industrial applicability (IA)	Yes: Claims	1-26
	No: Claims	-

**2. Citations and explanations (Rule 70.7):**

**see separate sheet**

**INTERNATIONAL PRELIMINARY  
REPORT ON PATENTABILITY  
(SEPARATE SHEET)**

PCT/CA2004/000998

The present application relates to embryonic stem (ES) cells obtainable from crosses between C57BL/6 and 129 inbred mouse (sub)strains. Some of said ES cells comprise a transgene docking site for introduction of a single copy introduction of a transgene. It has been shown that some of the ES cells derived from three inbred strains had the potential to give rise to chimeras with 100% ES cell derived coat color using the morula aggregation technique of generating embryos, indicating good development potential.

**Re Item I****Basis of the report**

Amended claims 4, 16 and 17, do not fulfil the requirement of Article 34(2)(b) PCT since no basis may be found for said amended claims:

In claim 4, the term 'X-linked' has been removed, whereas the description relates to X-linked HPRT.

Neither the description nor the claims as originally filed disclose a method for preparing mouse ES cells having good developmental potential, performing multiple generation of breeding including a combination of 'at least one cross and at least one backcross' (claims 16 and 17).

The amendments in claims 4, 16 and 17 have not been taken into consideration for this International Preliminary Examination Report.

**Re Item V****Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement****1) Reference is made to the following documents:**

D1: EGGAN KEVIN ET AL: "Hybrid vigor, fetal overgrowth, and viability of mice derived by nuclear cloning and tetraploid embryo complementation" PROC. NATL. ACAD. SCI. USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 98, no. 11, 22 May 2001 (2001-05-22), pages 6209-6214

- D2: YAGI TAKESHI ET AL: "A novel ES cell line, TT2, with high germline-differentiating potency" ANALYTICAL BIOCHEMISTRY, vol. 214, no. 1, 1993, pages 70-76
- D3: JASIN ET AL: "Targeted transgenesis" PROC. NATL. ACAD. SCI. USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 93, no. 17, August 1996 (1996-08), pages 8804-8808

**NOVELTY (Article 33(2) PCT)**

2.1) D1 discloses ES cells derived from various crosses between two different inbred mouse strains, including C57Bl/6 and a 129 substrain, and shows that said F<sub>1</sub> ES cell clones can give rise to embryos entirely (100%) derived from said ES cells, when injected into tetraploid blastocysts and subsequently transferred to recipient females (D1, table 4; page 6213, right hand column, paragraph 1). Some of the ES cells described were derived from mouse strains carrying a transgene docking site, the *Rosa26* locus, and mice have been generated from said cells after targeting of said *Rosa26* locus (D1, table 4).

It has been noted that D1 does not show that the ES cells derived from the hybrid mice compete successfully with pre-existing inner cell mass cells, and their derivatives, when injected into a normal blastocyst. However, there are no **technical features** that allow the skilled person to discriminate between the ES cells disclosed in D1 and the ES cells of claim 3 or 22, therefore they are considered to be implicitly identical.

If the ES cells disclosed in D1 are not suitable to practise the present invention, it seems that not all ES cells falling under the scope of claim 3 would solve the problem posed, hence said claim would lack support.

In conclusion, D1 anticipates the subject-matter of claims 3, 6-11 and 22-26 (Article 33(2) PCT).

2.2) In addition, claims 12-15 lack novelty because a product is not rendered novel merely by the fact that it is produced by means of a new process (PCT Guidelines A5.26[1]), and because there are no technical features present in claims 12-15 that allow the skilled person to distinguish between an ES cell derived mouse or transgenic mouse of said claims from (transgenic) mice obtained by crossing two or three inbred strains of mice known in the art (Article 33(2) PCT).

2.3) The subject-matter of claims 1, 2, 4, 5 and 16-21 is not disclosed in the prior art, therefore said claims are novel (Article 33(2) PCT).

**INVENTIVE STEP** (Article 33(3) PCT)

3.1) The document D2 is regarded as being the closest prior art to the subject-matter of claim 1, and discloses (D2, table 1): ES cells derived from a cross between two different inbred mouse strains, that give rise to chimeras that are 100% ES cell derived upon injection of the ES cells into eight-cell stage embryos.

From this the subject-matter of claim 1 differs in that **three** inbred (sub)strains have been used to generate ES cells having maximal heterosis and related development potential.

The problem to be solved by the present invention may therefore be regarded as the provision of further ES cells with maximal heterosis.

No surprising effect has been indicated of the use ES cells derived from a cross between three different inbred mouse (sub)strains compared to ES cells derived from a cross between two inbred mouse strains for the ability of said ES cells to contribute at a high percentage to the embryos generated from said ES cells. It is known in the art that heterozygous ES cells can give rise to entirely ES cell derived embryos (D1, table 4; D2, table 1) and the present application does not provide evidence for a **technical contribution** of the genetic information of a third inbred (sub)strain to heterosis and related development potential.

Therefore no inventive step may be acknowledged for the subject-matter of claim 1 (Article 33(3) PCT).

3.2) A method for preparing mouse ES cells having good development potential by mating a male of a first inbred strain with a female of a second inbred strain is well known in the art (D1, D2). From this, the subject-matter of claim 16 differs in that in addition multiple generations of breeding including crosses and backcrosses are performed with the offspring of aforementioned first mating.

The subject-matter of claim 16 thus provides a further method for preparing ES cells having good development potential.

The further breeding steps will change the relative contribution of the genetic information of

both inbred (sub)strains to the ES cells. However, the application fails to indicate what effect on the development potential of the ES cells is achieved by changing the ratio of the genetic information of both inbred (sub)strains, or what ratios would be suitable to practise the invention.

Similarly, no comparative example has been provided to demonstrate that a method of combining the genetic information derived from any three inbred (sub)strains (claim 17) results in ES cells with better development potential than the hybrid ES cells known in the art. Therefore, no technical effect has been demonstrated for the additional breeding steps or the introduction of genetic information from a third inbred (sub)strain, and no inventive step has been acknowledged for the subject-matter of claim 16 and 17.

**3.3)** Dependent claims 2, 4, 5 and 18-21 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, the reasons being as follows:  
the use of a specific sequence allowing introduction of a single copy insertion of a transgene in such a manner that does not disrupt endogenous genes, such as a deletion mutant of an X-linked hypoxanthine phosphoribosyltransferase gene or a sequence comprising a loxP site is well known (D3).

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